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Differential Cellular Requirements for Activation of Herpes Simplex Virus Type 1 Early (tk) and Late (gC) Promoters by ICP4

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The herpes simplex virus type 1 immediate-early protein, ICP4, activates the transcription of viral early and late genes and is essential for viral growth. It has been shown to bind DNA and interact with components of the general transcription machinery to activate or repress viral transcription, depending upon promoter context. Since early and late gene promoters have different architectures and cellular metabolism may be very different at early and late times after infection, the cellular requirements for ICP4-mediated activation of early and late genes may differ. This hypothesis was tested using tk and gC as representative early and late promoters, respectively. Nuclear extracts and phosphocellulose column fractions derived from nuclear extracts were able to reconstitute basal and ICP4-activated transcription of both promoters in vitro. When examining the contribution of the general transcription factors on the ability of ICP4 to activate transcription, the fraction containing the general transcription factor TFIIA was not essential for ICP4 activation of the gC promoter, but it was required for efficient activation of the tk promoter. The addition of recombinant TFIIA restored the ability of ICP4 to efficiently activate the tk promoter, but it had no net effect on activation of the gC promoter. The dispensability of TFIIA for ICP4 activation of the gC promoter required an intact INR element. In addition, microarray and Northern blot analysis indicated that TFIIA abundance may be reduced at late times of infection. This decrease in TFIIA expression during infection and its dispensability for activation of late but not early genes suggest one of possibly many mechanisms for the transition from viral early to late gene expression.

During the lytic cycle of herpes simplex virus type 1 (HSV-1) infection, synthesis of viral gene products occurs in three temporally regulated phases, immediate-early (IE or α), early (E or β), and late (L or γ) (42). Each gene contains its own promoter regulatory region and is transcribed by the cellular RNA polymerase II (Pol II) transcriptional machinery (1, 12). Each class of genes, however, differs with respect to its promoter structure, which decreases in complexity from IE to E to L genes (reviewed in references 85 and 87). These class-specific differences in promoter structure may be important in determining the ability to nucleate the assembly of stable preinitiation complexes at various phases of infection, in part mediating kinetic class-specific transcription (86, 92).

Five IE genes constitute the first set of genes to be transcribed upon HSV-1 infection. They are maximally expressed approximately 2 to 4 h postinfection (hpi) (42). These genes are expressed without prior viral protein synthesis due to the viral transactivator, VP16 (2, 6). VP16, a viral tegument protein, is released into the cell upon infection and associates with cellular proteins Oct1 and HCF to bind the virus-specific TA ATGARAT elements found exclusively in IE gene promoters to activate transcription from these promoters (28, 65, 66). In addition to a TATA box and TATTGARAT elements, sites exist for cellular *cis*-acting factors such as Sp1 and others that contribute to enhanced transcription (29).

Of the IE proteins, ICP4 is absolutely required for progression beyond the immediate-early phase of gene expression due to its role as a transcriptional activator of early and late genes (9, 16, 17, 20, 25, 32, 67, 68). As a transactivator, ICP4 functions to increase the rates of transcription by increasing the rate of transcription complex assembly on promoters (34). ICP4 interacts with components of the basal transcription apparatus to either activate or repress transcription (7, 35–37, 56, 79). Although ICP4 contains a DNA binding region that is essential to activation (18, 26, 64, 71, 75), no specific ICP4 binding sites have been identified on early and late promoters that are responsible for activation (24, 46, 78). Deletion of ICP4 severely impairs expression of early and late genes (16). However, certain mutations in ICP4 that have no effect on early gene transcription do not allow late gene expression, suggesting that ICP4 may act differently on early and late promoters (15, 18).

Efficient transcription of the β or early genes is strictly dependent on the presence of functional α proteins, particularly ICP4 (17, 20, 43, 49, 72, 73). Early promoters differ from IE promoters in that they lack the virus-specific TAATGARAT sequences present in IE promoters. However, they are similar to IE promoters in that they contain a TATA box and retain upstream cellular activating sequences, such as Sp1 and CCAAT boxes, that contribute to activation of these genes (24, 30, 81). Their expression peaks 4 to 6 hpi and is subsequently shut off.

The γ or late genes are the last set of genes that are ex-

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pressed. They are categorized as either leaky-late (γ1) or strict-late (γ2), depending on their requirement for DNA synthesis for expression. The γ1 genes can be suboptimally expressed in the absence of viral DNA synthesis, whereas the γ2, or true late genes, have a strict requirement for viral DNA synthesis (10, 40–42, 49). True late promoters are clearly distinct from IE and early promoters. These promoters are relatively simple in structure because, while they contain a TATA box, they are devoid of any essential upstream activating sequences (27, 39, 41, 44, 48). Only two known elements downstream from the TATA box are important for late gene expression, the initiator element (INR), which overlaps the transcriptional start site, and the downstream activating sequence (35, 38, 39, 44, 80). ICP4 has been shown to activate transcription from the true late gC promoter maximally when both a TATA box and INR are present (35). Mutations in the INR element of this promoter significantly reduce ICP4-activated transcription both in vitro and in vivo, indicating the importance of this element for late gene expression (54).

The basal RNA Pol II transcription machinery is composed of at least seven general transcription factors (GTFs), including TFIIA, -B, -D, -E, -F, and -H and Pol II, which assemble on core promoters in a coordinated manner or, as more recently proposed, as a preassembled holoenzyme complex (86). TFIID is a multiprotein complex containing a TATA-binding protein (TBP) and 8 to 12 tightly associated subunits known as TAFs or TBP-associated factors (21, 93). Binding of TFIID to the TATA box, via TBP, is critical for the rate and efficiency of preinitiation complex assembly (3, 91). In reconstituted transcription systems, TBP can function in the absence of TAFs to promote basal levels of transcription from TATA-containing core promoters (4). However, TBP alone is insufficient to support activated transcription by both viral and cellular activators, such as Sp1 VP16, and ICP4. Effective response to these activators requires TAFs in TFIID, and many activators have been shown to interact with TFIID through TAFs (reviewed in references 5, 31, and 33). TAFs also contribute to promoter selectivity by interacting with other basal transcription factors, other TAFs, and specific DNA sequences, such as the INR element (50, 51). TAF250 is an integral component of the TFIID complex that along with TAF150 and TBP has been shown to interact with the eukaryotic INR element (8, 52, 84). Interestingly, ICP4 was found to interact with TAF250 of TFIID through its C-terminal domain (7).

The GTF TFIIA binds TBP and stabilizes TBP binding to the TATA box (3, 11, 47, 57, 62). It is not required for basal transcription when reconstituted with TBP and the remaining GTFs. However, it has been shown that activator-enhanced transcription is not only mediated through the TAFs in TFIID but also is dependent on TFIIA (13, 55, 61, 69, 82, 89). TFIIA is composed of three subunits (α, β, and γ). The smallest subunit (γ) is required for the ability of TFIIA to stabilize TBP binding to the TATA box and is essential for activated transcription by upstream activators such as Sp1, VP16, NTF-1, and the Epstein-Barr virus activator Zta (13, 58, 70, 89). Mutations in the γ-subunit have been shown to disrupt transcriptional activation by VP16, GAL4-CTF, and AP-1 (69). However, in vitro transcription studies have shown that TFIIA is dispensable for activation of the HSV gC promoter by ICP4 (35).

The cellular requirements for activation of early promoters by ICP4 have not been studied. Given that HSV infection has a profound effect on cellular gene expression and metabolism, it is possible that the cellular transcription machinery is different at times when early and late genes are transcribed. To this end, we examined features of the general transcription machinery that contribute to ICP4 activation of two structurally distinct promoters, the early (tk) and the true late (gC) promoters, using a reconstituted in vitro transcription system. We showed that the GTF TFIIA was required for efficient ICP4 activation of the early tk promoter but was not essential for efficient ICP4 activation of the late gC promoter. The dispensability of TFIIA for activation of the gC promoter required a functional INR element. Addition of TFIIA greatly stimulated ICP4's ability to activate transcription of a mutated INR, suggesting that in the absence of an initiator element, activation by ICP4 requires the additional activities of TFIIA to stabilize TFIID binding to the promoter. In addition, we found that TFIIA subunit mRNA abundance was greatly reduced in cells late in infection. These data altogether suggest that at late times of infection the abundance of TFIIA may decline, potentially downregulating ICP4 activation from early promoters. Changes in the abundance of TFIIA would have little effect on the activation of true late promoters by ICP4, since they possess INR elements. This would allow for efficient expression of late genes while turning down expression of early genes.

MATERIALS AND METHODS

Cells and viruses. The KOS strain of HSV-1 was used to infect HEL cells for Northern blot analysis and Vero cells for ICP4 purification. HeLa cells were used to prepare nuclear extracts and phosphocellulose column fractions.

Preparation of phosphocellulose fractions, human and rTFIIA, and ICP4. Phosphocellulose fractions were prepared from 10 liters of HeLa nuclear extracts as previously described (7, 19). Approximately 15 ml of nuclear extract at 9.2 mg/ml was loaded onto a 15-ml packed column volume of phosphocellulose (P11) resin and eluted as previously described (19). Approximately 96 mg of total protein was eluted, 44% in the A fraction (42 mg in a 20-ml pool), 29% in the B fraction (28 mg in an 8-ml pool), 16% in the C fraction (16 mg in a 6-ml pool), and 11% in the D fraction (10 mg in a 6-ml pool). All fractions were dialyzed against D100 buffer (20 mM HEPES [pH 7.9], 10% glycerol, 100 mM KCl, 2 mM EDTA, 1 mM dithiothreitol, 2 mM phenylmethylsulfonyl fluoride) for use in transcription reactions. Human TFIIA was prepared from the first phosphocellulose fraction (the A fraction) by further fractionation on DEAE-Sephacel (7, 74). The 0.5 M KCl eluate (AB fraction) from this column, containing TFIIA, was ammonium sulfate precipitated (0.42 mg/ml), resuspended in buffer D100, and loaded over a Superose 12 gel filtration column equilibrated with D100, as described by Reinberg et al. to purify human TFIIA (74). Recombinant human TFIIA was produced from uncleaved α/β- and γ-subunits carrying histidine tags from overexpressing M15 bacteria harboring pQE-TFIIAαβ and pQE-TFIIAγ (70). The two polypeptides were independently purified on Ni-nitrilotriacetic acid–agarose columns under denaturing conditions and were renatured separately or in equimolar amounts to produce recombinant TFIIA (rTFIIA) as described elsewhere (55). ICP4 was purified from wild-type (KOS)-infected Vero nuclei as previously described (54). Purified rTFIIB used in transcription reactions and purified rTBP used in the gel shift analysis were previously described (7).

In vitro transcription and primer extension. The plasmids pLSWT (45), pgCLS1 (35), and pgCLS8 (54) were used as templates for in vitro transcription reactions. Reconstituted transcription and primer extension analysis reactions were carried out as previously described (54). Supercoiled DNA templates (100 ng) were incubated with nuclear extract or mixtures of phosphocellulose fractions in the presence or absence of approximately 250 ng of ICP4. Phosphocellulose fractions were added back based on their percent contribution to the total protein recovered from the phosphocellulose column to 55 μg per reaction mixture. For each reaction mixture, 11.5 μl of the A fraction, 4.6 μl of the B fraction, 3.4 μl of the C fraction, 3.2 μl of the D fraction, and 1 μl of a 0.31-mg/ml

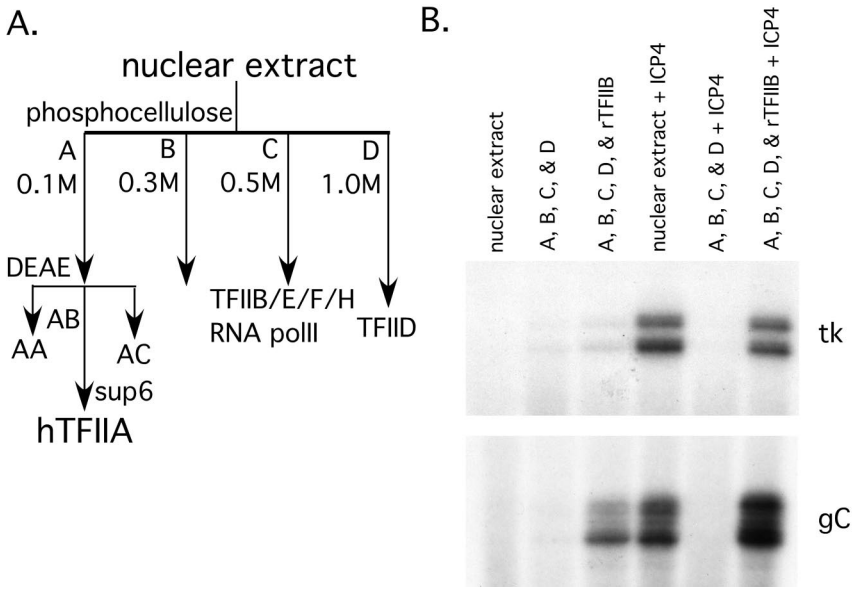

FIG. 1. Reconstitution of basal and ICP4-activated transcription from early (tk) and late (gC) promoters with phosphocellulose column fractions. (A) Scheme for transcription factor fractionation of nuclear extract on phosphocellulose resin column generating four distinct fractions (A, B, C, and D) that contain one or more of the basal transcription factors. The further fractionation of human TFIIA (hTFIIA) on DEAE and Superose 12 columns is also diagrammed. (B) The ability of phosphocellulose column fractions to reconstitute transcription on representative early and late promoters was assayed for in the presence and absence of purified ICP4. Each fraction was added back, based on the percent contribution to the total amount of protein recovered from the column, to equal 55 μg of protein per each reaction mixture and compared to 60 μg of nuclear extract. Shown are the autoradiographic images of primer extension products of the in vitro transcription reactions.

solution of rTFIIB was used. Where the A fraction was substituted, 3 μl of the AB fraction, 3 μl of Superose 12-purified TFIIA, or the indicated amount of rTFIIA was used.

Electrophoretic mobility shift assay. The EcoRI-BamHI fragment of the plasmid p4 (56) containing a TATA box and an ICP4 binding site was end labeled with ^{32}P using polynucleotide kinase, purified, and quantitated. DNA binding reaction mixtures (30 μl) contained 12.5 mM HEPES (pH 7.8), 60 mM KCl, 12.5% glycerol, 5 mM $MgCl_2$, 0.5 mg of bovine serum albumin/ml, 20 mM β-mercaptoethanol, 25 μg of poly(dG) · poly(dC)/ml, and the DNA probe (10^4 cpm) and were incubated with protein samples at 30°C for 30 min. The amounts of protein used in the binding reaction mixtures were 15 ng of TBP, 60 ng of rTFIIAαβ, or 15 ng of rTFIIAαβγ. After 30 min, 1 μl of anti-TFIIAγ was added where indicated and incubated for an additional 15 min at 30°C. Reactions were resolved on nondenaturing 4% 0.5× Tris-borate-EDTA–acrylamide gels and exposed to film.

Northern blot analysis. Poly(A)$^+$ RNA (2 μg) harvested from uninfected and KOS-infected (multiplicity of infection of 10) HEL cells at 4 and 8 hpi was resolved by denaturing formaldehyde-agarose gel electrophoresis, transferred to nitrocellulose membranes, and probed, washed, and exposed as previously described (22). ^{32}P-labeled probes were generated from pQE-TFIIAαβ and pQE-TFIIAγ (69) by nick translation using [α-^{32}P]dCTP and [α-^{32}P]dGTP.

RESULTS

Reconstitution of basal and ICP4-activated transcription on early (tk) and late (gC) promoters in vitro. It has previously been shown that nuclear extracts prepared from uninfected HeLa cells support basal and ICP4-activated transcription from both an early (tk) and a late (gC) promoter (35). When more-purified GTF systems are used instead of nuclear extracts, ICP4 efficiently activates the gC promoter (7, 35). However, ICP4-activated transcription of the tk promoter has not been tested in different GTF systems. To delineate any differences in requirements for cellular transcription factors for activation of early and late promoters, we first set out to examine the contribution of these factors in a crudely fractionated transcription system. The first step in a commonly utilized strategy for the purification of the GTFs from nuclear extract involves fractionation on phosphocellulose columns (Fig. 1A). Four fractions were generated, A, B, C, and D, depending on the salt concentration used for elution (0.1, 0.3, 0.5, and 1 M KCl, respectively). One or more of the GTFs can be found in each fraction (TFIIA is found in the A fraction, and TFIID is found in the D fraction). To reconstitute transcription, each fraction was added back as a percentage of the total protein amount recovered (see Materials and Methods). Equivalent amounts of protein were used in reconstitution experiments with both the tk and gC promoters, in the presence and absence of purified ICP4. As observed in Fig. 1B, both basal and ICP4-activated transcription levels of the tk and gC promoters were efficiently reconstituted using fractions A through D, with the addition of rTFIIB. Apparently, insufficient quantities of TFIIB were recovered in the C fraction to reconstitute transcription.

Since each phosphocellulose fraction was enriched for one or more of the basal transcription factors in addition to other factors that play a role in transcription, the contribution of each phosphocellulose fraction to basal and ICP4-activated transcription of both the tk and gC promoters was assessed (Fig. 2). Each fraction was individually omitted from the reaction mixtures with the tk and gC promoters, and transcription was assessed in the presence and absence of ICP4. The most striking result of this analysis was the differential requirement of the A fraction for ICP4 activation of the tk and gC promoters (Fig. 2). The A fraction was not required for efficient activation of the gC promoter by ICP4. In contrast, activation

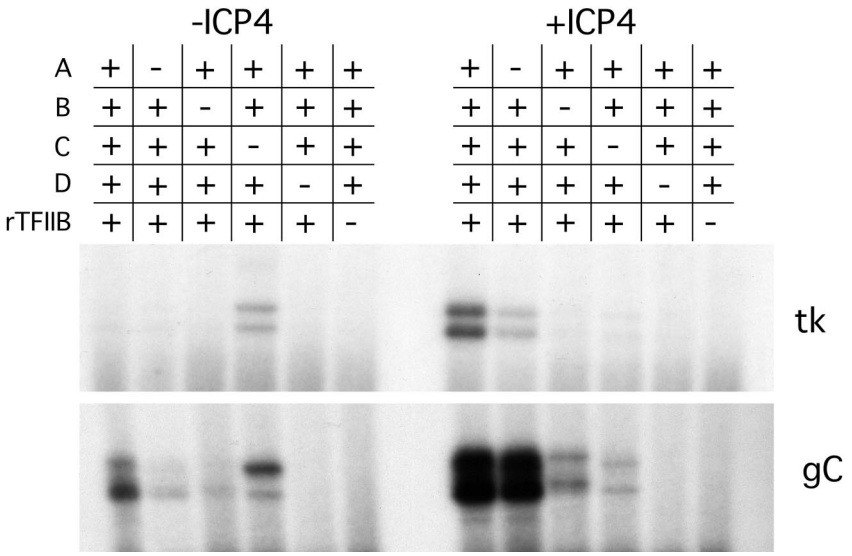

FIG. 2. Effect of individual phosphocellulose fractions on basal and ICP4 activation of the tk and gC promoters. The contribution of each phosphocellulose fraction to basal and ICP4-activated transcription was assayed on both early and late promoters by omitting each fraction from the in vitro transcription reaction in the presence and absence of ICP4. rTFIIB was also added where indicated.

of the tk promoter by ICP4 in the absence of the A fraction was very inefficient. This suggests that some factor or factors present in the A fraction are crucial for ICP4 activation of the tk promoter, but not for activation of the gC promoter.

Differential requirement for TFIIA in activation of the gC and tk promoters by ICP4. Because the GTF TFIIA is a component of the A fraction, we sought to further fractionate this factor to determine its relevance for activation of the tk promoter by ICP4. Human TFIIA was further fractionated by chromatography on a DEAE-Sephacel column. The bulk of TFIIA activity eluted in the 0.5 M KCl wash, also known as the AB fraction (Fig. 1A). To assess its contribution to ICP4 activation, the AB fraction was substituted for the A fraction in reactions with both tk and gC promoters (Fig. 3). In the absence of the A fraction, ICP4 efficiently activated the gC promoter, and the addition of AB had little effect on ICP4 activation. In contrast, omission of the A fraction highly diminished the ability of ICP4 to transactivate the tk promoter. Addition of the AB fraction, however, restored the ability of ICP4 to efficiently activate the tk promoter. These data suggest that this fraction, which is known to contain TFIIA, is important for activation of the tk promoter by ICP4 but is dispensable for activation of the gC promoter.

TFIIA was further purified from the AB fraction by gel filtration on Superose 12 (Fig. 1A). Fractions from the Superose 12 column were tested for the ability to complement ICP4 activation of the early tk promoter (Fig. 4A). Complementing activity was most abundant in fractions 6 and 7, which also corresponded to a molecular mass of about 82 kDa, in accordance with the previously published molecular mass of the TFIIA complex and its elution profile on Superose 6 (74). The ability of this form of TFIIA to support activation of the tk promoter was compared to that of the AB fraction and found to substitute as well as or better than the AB fraction in restoring the ability of ICP4 to activate the tk promoter (Fig. 4B). This indicated that TFIIA found in the A fraction was the

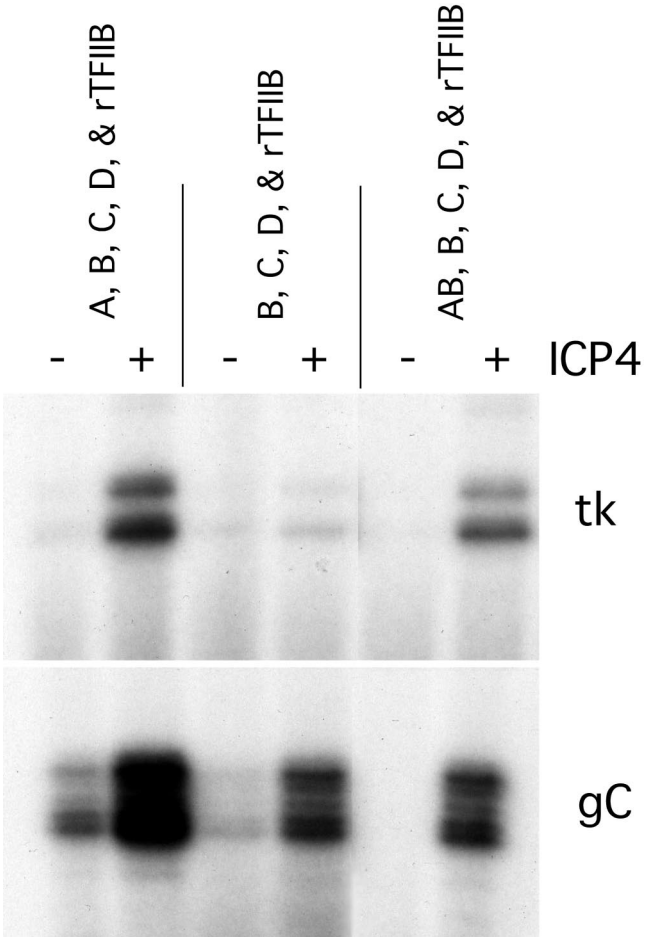

FIG. 3. Effect of the AB fraction on the ability of ICP4 to efficiently activate an early (tk) promoter and a late (gC) promoter. The phosphocellulose A fraction was further fractionated on DEAE-Sephacel (Fig. 1A), and the 0.5 M KCl wash (AB fraction) containing the GTF TFIIA was tested for its ability to substitute for the A fraction in ICP4 activation.

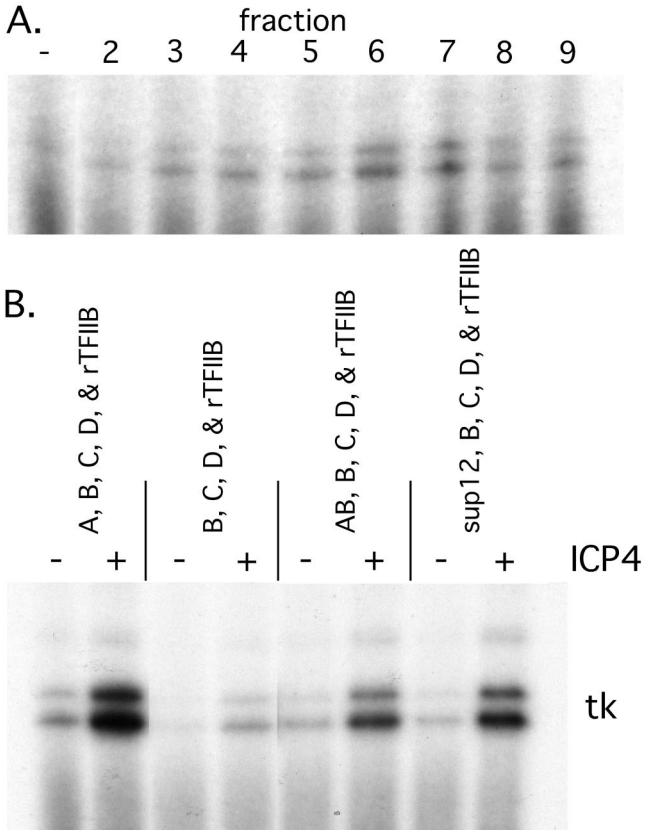

FIG. 4. Complementation of ICP4 activation of the tk promoter by Superose 12 fractions. (A) Complementing activity was further purified from the AB fraction of DEAE-Sepharose by Superose 12 gel filtration. Each fraction was assayed for its ability to support ICP4 activation from the tk promoter in vitro. (B) Superose 12-purified TFIIA was substituted for the A or AB fraction in reconstituted in vitro transcription reactions, with or without ICP4.

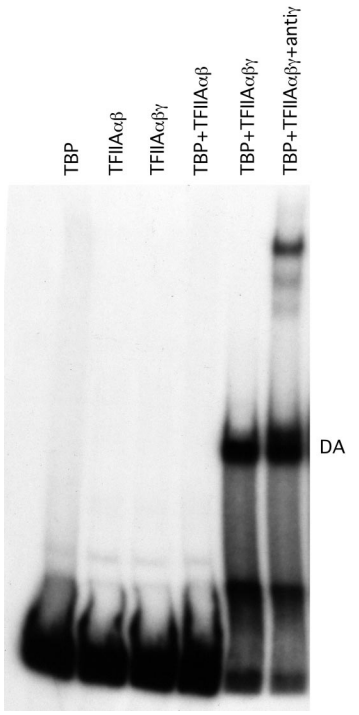

FIG. 5. Reconstitution of TFIIA activity using recombinant proteins. TFIIAαβ and TFIIAγ were expressed in *Escherichia coli*, purified under denaturing conditions, and renatured separately or together. rTFIIA activity was assessed via gel mobility shift analysis with αβ or αβ+ γ and TBP on the TATA motif of the ICP4 promoter.

factor responsible for efficient activation of the tk promoter by ICP4.

To strengthen the interpretation that TFIIA was crucial for activation of tk and to rule out the possibility that some factor copurifying with TFIIA was contributing to the stimulatory effect on the tk promoter, rTFIIA was produced and used as an alternate source. Human TFIIA is comprised of three subunits, α, β, and γ, which have sizes of 35, 19, and 13 kDa, respectively. The α- and β-subunits of the human form are encoded by one gene (14, 61, 88), while the γ-subunit is encoded on a separate gene (13, 70, 82, 89). rTFIIA was produced from bacteria separately overexpressing TFIIAαβ and TFIIAγ, purified under denaturing conditions, and renatured either separately or in a mixture of equal molar amounts. Gel mobility shift assays were conducted on a DNA fragment containing a TATA box to assess the activity of reconstituted rTFIIA (Fig. 5). TBP alone failed to bind the probe, as did either TFIIAαβ or TFIIAαβγ. TBP plus TFIIAαβ also failed to bind the probe, due to the absence of the γ-subunit, which is required for the stabilized binding of TBP to TATA boxes. However, TFIIAαβγ stabilized the binding of TBP to DNA, forming the previously observed DA complex (13, 70, 82, 89). An antibody specific to the γ-subunit was able to induce a supershift, suggesting the presence of TFIIA in this complex and confirming the successful reconstitution of TFIIA activity.

rTFIIA was then compared to the A and Superose 12 fractions for the ability to support ICP4-activated transcription of the tk and gC promoters in vitro (Fig. 6). Again, ICP4 efficiently activated the gC promoter independent of a TFIIA-containing preparation and was unable to efficiently activate the tk promoter in the absence of a TFIIA preparation (Fig. 6A). However, rTFIIA, like the A and Superose 12 fractions, restored the ability of ICP4 to efficiently activate the tk promoter. These data support the interpretation that TFIIA is not essential for activation of the late gC promoter but is required for efficient activation of the early tk promoter.

True late promoters are relatively simple promoters that do not require upstream activating sequences for efficient expression (85, 87). The efficient activation of the gC promoter by ICP4 requires only an intact TATA box and INR region. The initiator element has been shown to be crucial for ICP4's ability to activate the gC promoter, since mutations in the initiator reduce its ability to efficiently activate transcription (35, 54). TFIID makes contact with the INR, and ICP4 facilitates TFIID binding to promoters (7, 34). TFIIA has previously been shown to be essential for activated transcription in the context of TFIID, yet as our results indicate, TFIIA is not essential for ICP4 to activate the gC promoter. TFIIA has also been shown to stabilize TFIID binding to the TATA box. Therefore, we reasoned that it was possible that in the absence of a functional INR, TFIIA might restore the ability of ICP4 to activate transcription. To this end, we compared the ability of

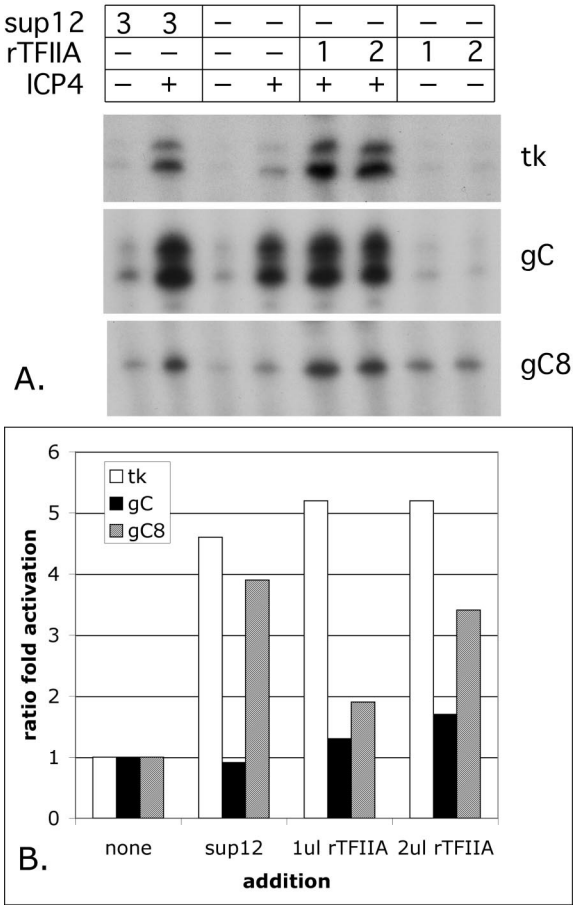

FIG. 6. Ability of rTFIIA to enhance ICP4 activation of an early (tk), a late (gC), and an initiator-mutated late (gC8) promoter in vitro. (A) The indicated amounts (in microliters) of Superose 6 human TFIIA and rTFIIA were assayed for the ability to support ICP4 activation of the tk, gC, and gC8 promoters in vitro. Shown are the autoradiographic images of primer extension products of the in vitro transcription reactions. (B) Quantification of in vitro transcription reactions conducted as described in the legend for panel A. Additional in vitro transcription reactions were conducted precisely as described for panel A. The dried gel containing the electrophoretically separated primer extension products was analyzed using a Storm 840 PhosphorImager. Under each condition, the fold activation by ICP4 from two determinations was calculated and averaged. Without the addition of a TFIIA-containing fraction, ICP4 activated the tk, gC, and gC8 promoters by five-, nine-, and fourfold, respectively. The values for fold activation under each of the conditions (TFIIA additions) were divided by fold activation for no addition in reactions with the corresponding promoter. Shown are the ratios of fold activation for no addition (none), Superose 12 fraction addition (sup12), and the addition of the indicated amounts of rTFIIA.

ICP4 to activate the gC promoter to that of a derivate of the gC promoter with a mutated INR element, gC8, in the presence and absence of TFIIA (Fig. 6A). gC8 has three nucleotides mutated in the initiator region and is severely compromised for ICP4 activation (54). In the absence of the A fraction, gC8 was minimally activated by ICP4, and addition of the A fraction somewhat stimulated activation. Substitution of the A fraction with human TFIIA or rTFIIA significantly stimulated ICP4 activation, suggesting that in the presence of TFIIA the requirement for the INR element for activation by ICP4 is reduced.

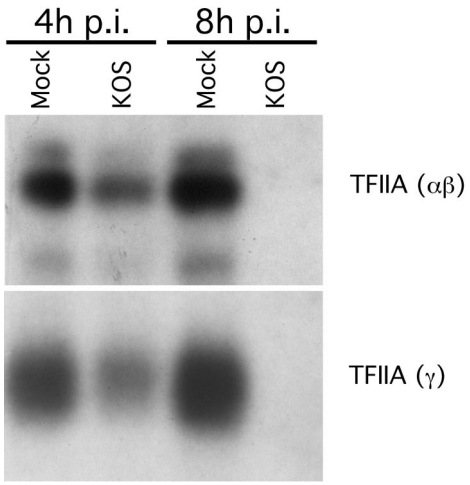

FIG. 7. TFIIA mRNA levels as a function of infection. Poly(A)$^+$ RNA (2 μg) isolated from KOS-infected (multiplicity of infection = 10) or mock-infected HEL cells at 4 and 8 hpi was subjected to Northern blot analysis probing for all the TFIIA subunit mRNAs.

A quantitative representation of the effects of TFIIA on ICP4 activation of the tk, gC, and gC8 promoters is given in Fig. 6B. Additional reactions were conducted as described for Fig. 6A. The fold activation under each condition was determined as described in the figure legend. Shown are the ratios for fold activation with the indicated addition relative to that with no addition. Therefore, while gC was strongly activated by ICP4 without a TFIIA addition (Fig. 6A), the addition of TFIIA had little effect on the fold activation (Fig. 6B). In contrast, the addition of the Superose 12 TFIIA preparation to reaction mixtures with the tk and gC8 promoters resulted in 4.6- and 3.9-fold-greater activation, respectively, compared to that in reactions without TFIIA addition. Similar results were obtained upon the addition of rTFIIA (Fig. 6B).

Expression of TFIIA during wild-type HSV infection. Because cellular gene expression is significantly changed during the course of lytic infection, we were interested in looking at what consequences this had on the expression of components of the basal transcription machinery as a function of time postinfection. Microarray analysis conducted on mock-infected cells and cells infected with wild-type virus for 4 and 8 h revealed a decrease in the abundance of the mRNA for the smallest γ-subunit of TFIIA (data not shown). Northern blot analysis using 2 μg of poly(A)$^+$ RNA from mock- and KOS-infected cells confirmed the decreased expression of this subunit as well as that for the other subunits of TFIIA. By 8 hpi, TFIIA mRNA was undetectable (Fig. 7). These data indicate that TFIIA abundance may decrease as a function of infection.

DISCUSSION

Unlike many cellular activators, ICP4 does not have a requirement for specific DNA binding sites for activation, and it can therefore activate transcription from a variety of promoters. Looking at two distinct promoters, the tk and the gC promoters, we found different cellular requirements for ICP4-

activated transcription. Specifically, the GTF TFIIA was required for efficient ICP4 activation of the tk promoter but was not essential for activation of the late (gC) promoter. These results indicate that ICP4 has some different requirements for components of the general transcription machinery, based on the promoter and possibly on the HSV kinetic class of promoter.

These results suggest that the composition or the assembly of ICP4-activated transcription complexes on the two promoters is different. In the assembly of Pol II transcription complexes, TFIID is the first factor recruited to the TATA box and is required for subsequent complex assembly (reviewed in references 3, 83, and 90). By participating in the complex, TFIIA helps stabilize binding of TFIID to the TATA box (11, 47, 57, 62). TFIIA and the TAFs present in TFIID are also required for activated transcription by such activators as VP16, Sp1, CCAAT binding factor, and the Epstein-Barr virus transactivator Zta (59, 69, 89). In addition, many of these activators have been shown to interact with TFIIA, TFIID, or both, and these interactions correlate with the ability to stabilize or enhance TFIID-TFIIA-promoter complex assembly (55, 58, 70) and promote transcription. In contrast, ICP4 will activate the gC promoter in the absence of TFIIA (7, 35) (Fig. 6B). It has also been shown to interact with TFIID (7), enhance the binding of TFIID to TATA boxes, and promote preinitiation complex formation in the absence of TFIIA (34). Therefore, it is likely that ICP4 operationally substitutes for TFIIA in that it independently performs a function normally associated with TFIIA, allowing for efficient activation of the gC promoter.

TFIIA, however, is clearly required for efficient activation of the tk promoter. One difference between the tk and gC promoters is the existence of an INR element located at the start site of gC transcription. Core promoters contain a TBP sequence, located 25 to 30 bp upstream of the transcription start site, and some have an initiator element (INR) encompassing the start site. The presence of an INR in addition to a TATA box can serve to enhance the strength of that promoter (23, 60). For promoters that lack a TATA consensus, the initiator element can direct accurate transcription initiation by itself or in conjunction with other promoter elements (63, 76, 77). TAF150, TAF250, and TBP found in TFIID have been shown to stably interact with the INR element and are sufficient for INR activity (8, 84). ICP4 has been shown to bind TFIID through TAF250 and has been shown to stabilize TFIID interaction to the core promoter (7). The late gC promoter contains both a TATA box and an INR region. Mutations in the initiator element diminish the ability of ICP4 to activate transcription (35, 54). We show here that in the context of an intact INR element, ICP4 does not require TFIIA for activated transcription (Fig. 6). This suggests that ICP4 sufficiently stabilizes TFIID through the INR element without the activities of TFIIA. In the presence of a mutated INR, ICP4 alone is no longer able to stabilize TFIID interaction and requires the additional activities of TFIIA. In the presence of TFIIA, ICP4 overcomes the requirement for an intact INR and in conjunction with TFIIA stabilizes TFIID-promoter interactions, thereby promoting transcriptional activation by ICP4. Although ICP4 interacts with TFIID through TAF250 and TFIIA interacts with TFIID through TBP, it is not currently known whether ICP4 directly interacts with TFIIA. It will be interesting to determine whether ICP4 participates in a tripartite complex with TFIIA and TFIID (or TBP) on early and late promoters and to determine the contribution of the INR element on these potential interactions.

Biological significance of the differential requirement for TFIIA in activation of early and late genes. HSV infection results in dramatic cellular changes, occurring at the levels of mRNA and protein abundance and posttranslational modification. At late times of infection, the expression of cellular proteins is greatly perturbed, suggesting that the composition and/or abundance of components of the Pol II transcriptional machinery may also be significantly altered. Northern blot analysis indicated that the expression of the smallest subunit of TFIIA decreased as a function of infection (Fig. 7). The viral functions that downregulate TFIIA expression are not currently known. However, this result suggests that TFIIA may not be as available for transcription late after infection. This of course would also depend on the stability and activity of the preexisting three-peptide subunits of TFIIA.

While INR elements have been found on most late promoters, they have not been found or described in early promoters. Therefore, it is possible that a reduction in TFIIA abundance may be one of the mechanisms functioning in the switch from early to late transcription. It is likely that this mechanism is not the only one functioning in this switch. Sp1, which is clearly involved in the transcription of early genes and immediate-early genes, is phosphorylated about the time early genes are shut off. This phosphorylated form of Sp1 is less active in transcription than uninfected cell Sp1 (53). Viral DNA replication is also involved in the switch from early to late gene transcription. In addition, TFIIA is required for activated transcription by VP16, Sp1, and the CCAAT binding protein. Since these activators are involved in immediate-early and early gene transcription, a reduction in TFIIA activity would also reduce the effect of these activators on immediate-early and early transcription.

ICP4 is a large complex molecule possibly with many features and functions that have yet to be recognized that allow it to activate transcription from a variety of promoters. In the context of lytic infection, it would follow that if components of the transcription machinery were changing as a function of infection, ICP4 might activate transcription by a variety of mechanisms involving different contacts with different cellular transcription factors. As presented here, TFIIA is required for efficient activation of early promoters but not late promoters. The decreased abundance of TFIIA late in infection may contribute to the switch from early to late transcription.

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